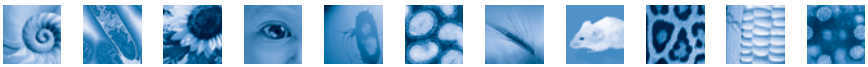
NimbleGen Arrays User's Guide

Gene Expression Analysis

For life science research only.



HIGH - DEFINITION GENOMICS

NimbleGen Arrays User's Guide

Gene Expression Analysis

For life science research only.

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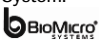

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Chapter 1. Before You Begin

This user's guide describes the processes for analyzing gene expression using these NimbleGen array formats:

- 385K (385,000 probes)
- 4x72K (4 x 72,000 probes)
- 12x135K (12 x 135,000 probes)

The first step involves creating cDNA from eukaryotic total RNA or poly-A^+ RNA (mRNA) and cDNA from prokaryotic total RNA using the Invitrogen SuperScript Double-Stranded cDNA Synthesis Kit. The second step requires labeling the cDNA using a NimbleGen One-Color DNA Labeling Kit and subsequent hybridization to a NimbleGen array using a NimbleGen Hybridization System. Subsequent steps include washing, drying, and scanning the array. Finally, data is extracted from the scanned image and analyzed for gene expression levels.

What's New?

Version 3.0 of this user's guide includes instructions on using NimbleGen 12x135K arrays, which enable you to hybridize up to 12 independent samples on a single slide, for gene expression experiments. It also includes details about the newly configured NimbleGen kits for array hybridization and processing (refer to pages 6 - 7 for more information on the available kits).

Version 3.1 describes how to seal the fill and vent ports of HX12 mixers using mixer multi-port seals. As of October 2008, mixer multi-port seals are included with HX12 mixers instead of mixer port seals. Refer to page 29 for instructions. This version also includes details about the experimental metric features available in the NimbleScan v2.5 software and a troubleshooting chapter.

Version 3.2 includes instructions on how to process samples using the NimbleGen One-Color DNA Labeling Kit and NimbleGen Microarray Dryer.

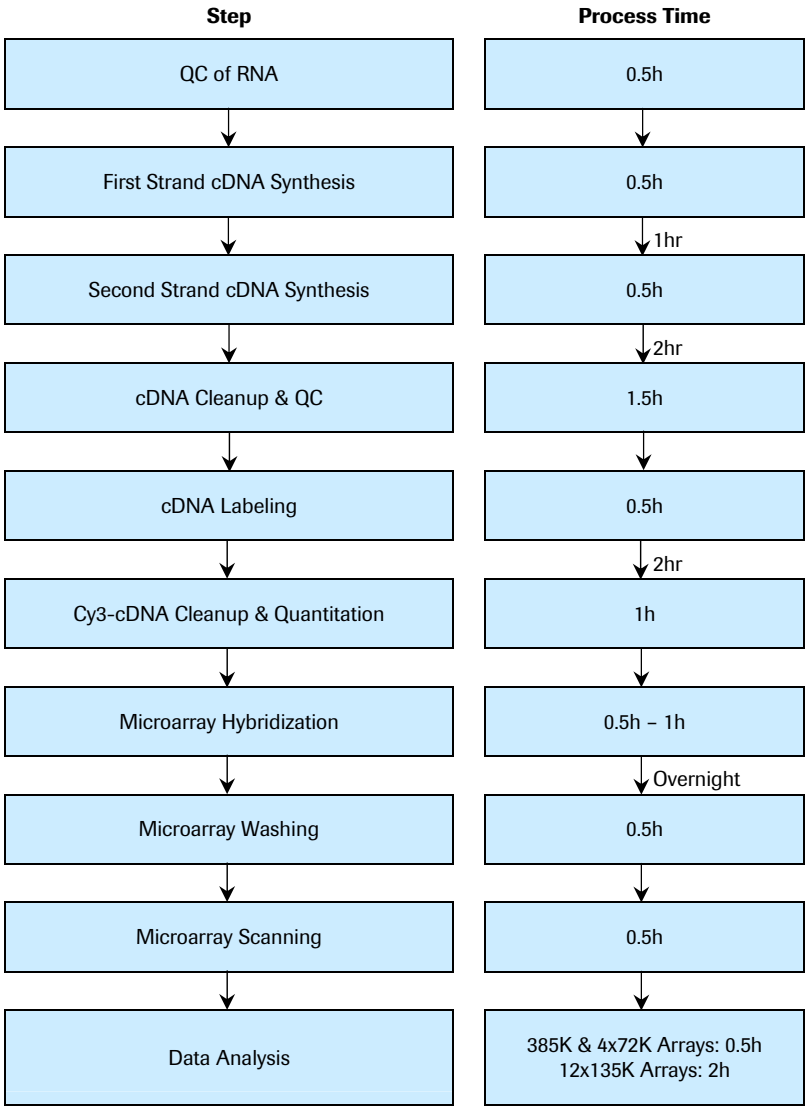


Figure 1: Workflow for NimbleGen Gene Expression Analysis. Steps in the process and estimated time for each step, based on the processing of one slide, are shown in the boxes. Incubation times are indicated beneath the appropriate process times.

Components Supplied

Component	385K Array	4x72K Array	12x135K Array
NimbleGen Arrays		As ordered	
NimbleGen Mixers	One X1 mixer is ordered separately from the 385K array	One X4 mixer is provided per 4x72K array	One HX12 mixer is provided per 12x135K array
Mixer Port Seals or Mixer Multi-port Seals	Mixer port seals are provided with X1 mixers	Mixer multi-port seals are provided with X4 mixers	Mixer multi-port seals are provided with HX12 mixers
NimbleGen Arrays User's Guide: Gene Expression Analysis		One booklet	
NimbleGen Design Files		One CD/DVD	

Microarray Storage

Store NimbleGen microarrays in a desiccator at room temperature until ready to use.

Protocol Information & Safety

- Wear gloves and take precautions to avoid sample contamination.
- To avoid RNA degradation, Roche NimbleGen recommends using RNase ZAP to eliminate contamination from work surfaces.
- Keep RNA on ice at all times.
- Keep RNase A in a designated work area and use designated pipettes and tips to prevent RNase A contamination throughout the lab. Change gloves after handling RNase A and clean work area with RNase ZAP immediately after use.

- Phenol:chloroform:isoamyl alcohol is dangerous and should be handled with caution. Wear the correct personal protective equipment when handling this chemical. Refer to MSDS for more information.
- Cy dyes are light sensitive. Be sure to minimize light exposure of the dyes during use and store in the dark immediately after use.
- Cy dyes are ozone sensitive. Take the necessary precautions to keep atmospheric ozone levels below 20ppb (parts per billion).
- Cy dyes are humidity sensitive. Take the necessary precautions to keep humidity levels below 40%.
- Roche NimbleGen has found that using VWR water and DTT for all post-hybridization washes results in higher signal from Cy dyes.
- Roche NimbleGen recommends using a NanoDrop Spectrophotometer for quantifying and characterizing nucleic acid samples because this instrument requires only 1.5µl of sample for analysis.
- Perform all centrifugations at room temperature unless indicated otherwise.

Required Equipment, Labware & Consumables

You assume full responsibility when using the equipment, labware, and consumables described below. These protocols are designed for the specified equipment, labware, and consumables.

NimbleGen Hybridization System

Choose between the following:

Equipment	Supplier	Process Quantity	Item Number
NimbleGen Hybridization System 4*	Roche NimbleGen	4 slides	05223652001 (110V)
			05223679001 (220V)
NimbleGen Hybridization System 12*	Roche NimbleGen	12 slides	05223687001 (110V)
			05223695001 (220V)

* NimbleGen Hybridization Systems include an accessory kit that contains a Precision Mixer Alignment Tool (PMAT), Mixer Disassembly Tool, Mixer Brayer, System Verification Assemblies, replacement O-rings, and forceps.

Microarray Drying System

Equipment	Supplier	Process Quantity	Item Number
NimbleGen Microarray Dryer	Roche NimbleGen	24 slides	05223636001 (110V)
			05223644001 (220V)

Scanner

Equipment	Supplier	Item Number
GenePix 4000B	Molecular Devices	NA

Software

Program	Supplier	Item Number
NimbleScan v2.5	Roche NimbleGen	05225035001 (Individual License)
		05225043001 (Site License)
ArrayStar® v2.1 (trial version)	Roche NimbleGen	05225060001

Standard Laboratory Equipment

Equipment	Supplier	Item Number
NimbleGen Compressed Gas Nozzle	Roche NimbleGen	05223628001
Bioanalyzer	Agilent	2100
Desiccator	*Multiple Vendors*	
Heat block (capable of temperatures to 98°C)	*Multiple Vendors*	
Microcentrifuge (12,000 x g capability)	*Multiple Vendors*	
Microman M10 Pipette (recommended for 4x72K and 12x135K arrays)	Gilson	F148501
Microman M100 Pipette (recommended for 385K arrays)	Gilson	F148504
Spectrophotometer	NanoDrop	ND-1000
SpeedVac	Thermo Savant	
Thermocycler	*Multiple Vendors*	
Vortex Mixer	*Multiple Vendors*	

Consumables & Accessories from Roche NimbleGen

Component	Package Size / Process Quantity	Item Number
NimbleGen X1 Mixer (for 385K arrays; includes mixer port seals)	10 mixers	05223725001
NimbleGen X4 Mixer (for 4x72K arrays; includes mixer multi-port seals)	10 mixers	05223733001
NimbleGen HX12 Mixer (for 12x135K arrays; includes mixer multi-port seals)	10 mixers	05223768001
NimbleGen One-Color DNA Labeling Kit	20 Cy3 labeling reactions	05223555001
NimbleGen Hybridization Kit	■ 100 hybridizations using X1 mixers ■ 160 hybridizations using X4 mixers ■ 120 hybridizations using HX12 mixers	05223474001

Component	Package Size / Process Quantity	Item Number
NimbleGen Hybridization Kit, LS (Large Scale)	■ 300 hybridizations using X1 mixers ■ 480 hybridizations using X4 mixers ■ 480 hybridizations using HX12 mixers	05223482001
NimbleGen Sample Tracking Control Kit	120 hybridizations for 4x72K arrays or 12x135K arrays	05223512001
NimbleGen Wash Buffer Kit	10 washes (processing 4 slides per wash)	05223504001
NimbleGen Array Processing Accessories		05223512001

Contents of NimbleGen Kits & Accessories

Kit	Contents
NimbleGen One-Color DNA Labeling Kit	■ Nuclease-free Water (2 x vial 1) ■ Random Primer Buffer (vial 2) ■ Cy3-Random Nonamers (vial 3) ■ Klenow Fragment (3'->5' exo-) 50U/µl (vial 4) ■ 10mM dNTP Mix (vial 5) ■ Stop Solution (0.5M EDTA) (vial 6) ■ 5M NaCl (vial 7)
NimbleGen Hybridization Kit and NimbleGen Hybridization Kit, LS	■ 2X Hybridization Buffer ■ Hybridization Component A ■ Alignment Oligo[1]
NimbleGen Sample Tracking Control Kit	■ Sample Tracking Controls[2]
NimbleGen Wash Buffer Kit	■ 10X Wash Buffer I ■ 10X Wash Buffer II ■ 10X Wash Buffer III ■ 1M DTT

Kit	Contents
NimbleGen Array Processing Accessories	■ Slide Rack ■ Wash Tanks ■ Slide Containers

1 The Alignment Oligo is a mixture of Cy3- and Cy5-labeled 48mer oligonucleotides that hybridize to alignment features on NimbleGen arrays. It is required for proper extraction of array data from the scanned image.

2 Twelve Sample Tracking Controls (STCs) are provided. Each STC is a Cy3-labeled 48mer oligonucleotide. When a unique STC is added to each sample before hybridization to a multiplex array, the STC can be used to confirm that the correct sample was hybridized to each array.

Reagents/Consumables from Other Vendors

Component	Supplier	Package Size	Item Number
β-Mercaptoethanol	Sigma Aldrich	25ml	M3148
0.5M EDTA	Sigma Aldrich	100ml	E7889
5mg/ml Glycogen	Ambion	5 x 1ml	9510
7.5M Ammonium Acetate	Sigma Aldrich	1 liter	A2706
Absolute Ethanol	Sigma Aldrich	500ml	E702-3
Compressed Inert Gas*	*Multiple Vendors*		
CP10 Pipette Tips (for 4x72K and 12x135K arrays)	Gilson	192 tips	F148412
		960 tips	F148312
CP100 Pipette Tips (for 385K arrays)	Gilson	192 tips	F148414
		960 tips	F148314
Isopropanol	Sigma Aldrich	500ml	I-9516
Oligo dT Primer	*Multiple Vendors*		
Phase Lock tubes (Light 1.5ml)	Fisher Scientific	200 tubes	FP2302800
Phenol:chloroform: isoamyl alcohol (25:24:1)	Ambion	100ml	9730
Random Primer	*Multiple Vendors*		
RNase A Solution	Promega	4mg/ml	A7973

Component	Supplier	Package Size	Item Number
SuperScript Double-Stranded cDNA Synthesis Kit	Invitrogen	10 reactions	11917-010
		50 reactions	11917-020
Water: reagent grade, ACS, nonsterile, type 1	VWR	2.5 gallon	RC915025
Cotton Swabs	*Multiple Vendors*		

* Roche NimbleGen recommends using the NimbleGen Compressed Gas Nozzle to gently blow compressed inert gas across arrays to remove any dust or debris. The use of canned aerosol compressed air for this purpose is not recommended and could compromise array and data quality.

Technical Support

If you have questions, contact your Roche NimbleGen Account Manager or Roche Microarray Technical Support. Go to *www.nimblegen.com/arraysupport* for contact information.

Notes

Chapter 2. Sample Preparation & QC

Chapter 2 describes how to create cDNA from eukaryotic total RNA or poly-A+ RNA (mRNA), and cDNA from prokaryotic total RNA using Invitrogen SuperScript Double-Stranded cDNA Synthesis Kit.

Sample Requirements

- High-quality RNA is required for optimal cDNA synthesis yield and cDNA labeling for microarray hybridization. Suggested RNA isolation methods successfully used with the NimbleGen Gene Expression platform are the Qiagen RNeasy® Kit (Catalog No. 74104) and Invitrogen TRIzol® reagent (Catalog No. 15596-026).
- Roche NimbleGen recommends starting with the following amounts of total RNA or poly-A+ RNA amounts for each hybridization:

Array Format	Eukaryotes	Prokaryotes
385K arrays	10µg total RNA 1µg poly-A+ RNA	10µg total RNA
4x72K arrays	10µg total RNA 1µg poly-A+ RNA	10µg total RNA
12x135K arrays	10µg total RNA 1µg poly-A+ RNA	10µg total RNA

Step 1. Spectrophotometric QC of RNA

Prior to synthesizing cDNA, verify that the RNA samples are of sufficient purity to proceed with cDNA synthesis.

1. Quantitate each RNA sample according to the following formula:

   $$\text{RNA Concentration } (\mu g/ml) = A_{260} \times 40 \times \text{Dilution Factor}$$

   RNA samples must have a concentration $\geq 1.0 \mu g/\mu l$.

2. Verify all samples meet the following requirements:

   - $A_{260}/A_{280} \geq 1.8$
   - $A_{260}/A_{230} \geq 1.8$

Step 2. Bioanalyzer/Gel QC of RNA

Verify RNA samples are of sufficient molecular weight. Roche NimbleGen recommends the use of an Agilent 2100 Bioanalyzer, because the required sample size is very small. If a Bioanalyzer is not available, use a denaturing agarose gel.

1. Transfer 250ng total RNA or 250ng poly-A^+ RNA to a sterile microcentrifuge tube. Store the remainder of your sample on ice or at -80°C.

2. Analyze samples using the Agilent Bioanalyzer and RNA 6000 Nano Assay Reagent Kit.

3. Compare the Bioanalyzer traces to those traces in Figures 2 and 3. Degraded samples appear as significantly lower intensity traces with the main peak area shifted to the left and typically exhibit much more noise in the trace.

   Note: *If using a denaturing agarose gel, one reagent source is Ambion NorthernMax® reagents for northern blotting. This kit's first section includes reagents and instructions for using denaturing agarose gels. You can also purchase reagents individually. Compare gel images to the Bioanalyzer electropherogram images.*

   Samples exhibiting degradation should not be carried through labeling and hybridization because there is an unacceptable risk of poor results.

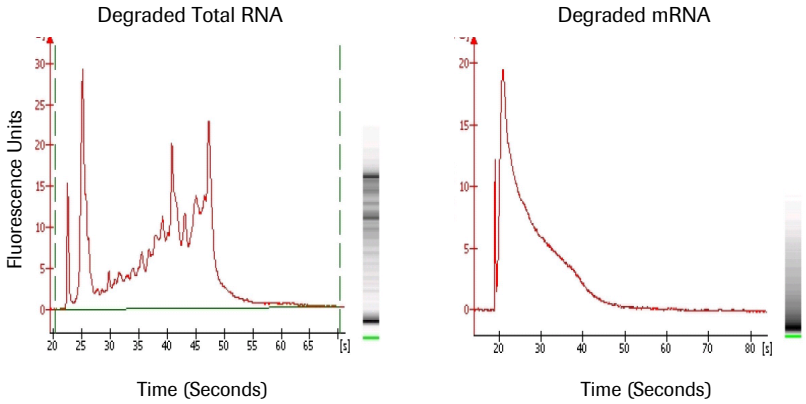


Figure 2: Example Traces for Degraded RNA Samples from a Eukaryotic Organism

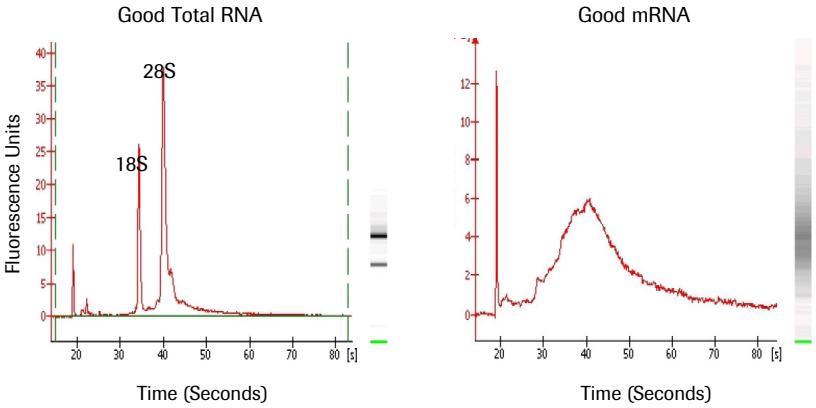


Figure 3: Example Traces for Non-degraded RNA Samples from a Eukaryotic Organism

Note: *Your traces could look different than these examples. Compare your traces to reference traces for the organism being researched.*

Step 3. First Strand cDNA Synthesis

Use the Invitrogen Superscript Double-Stranded cDNA Synthesis Kit to synthesize double-stranded cDNA.

1. Thaw and maintain the following components on ice. Combine components in a 0.2ml tube on ice according to the following tables.

   Note: *Roche NimbleGen does not recommend a vendor for oligo dT or random hexamer primer. Follow your preferred vendor's recommendation for the amount of primer to use. For example, Invitrogen recommends using 1µl of 100pmol/µl primer.*

Eukaryotic Source of RNA	Total RNA Amount	Poly-A+ RNA (mRNA) Amount
RNA	10µg	1µg
oligo dT Primer	1µl	1µl
DEPC Water	To volume	To volume
Total	**11µl**	**11µl**

Prokaryotic Source of RNA	Total RNA Amount
RNA	10µg
Random Hexamer Primer	1µl
DEPC Water	To volume
Total	**11µl**

2. Heat sample(s) to 70°C for 10 minutes in a thermocycler. Briefly spin tubes in a microcentrifuge and place them in an ice-water slurry for 5 minutes.

3. Add the following to each sample tube. (You can use a master mix when preparing multiple samples.)

Component	Volume
Reaction volume from step 2	11µl
5X First Strand Buffer	4µl
0.1M DTT	2µl
10mM dNTP Mix	1µl
Total	**18µl**

4. Mix gently (avoid vortexing). Briefly spin the tube(s) in a microcentrifuge.
5. Place sample(s) in a thermocycler set at 42°C for 2 minutes.
6. Add 2µl of SuperScript II and mix gently (avoid vortexing).
7. Incubate the sample(s) at 42°C for 60 minutes.
8. **STOP POINT:** Briefly spin the tube(s) in a microcentrifuge. Place the sample(s) on ice until the second strand synthesis. You can store the sample(s) overnight at -20°C.

Step 4. Second Strand cDNA Synthesis

1. Add the following components to the first strand reaction(s) in the indicated order; you can use a master mix. Keep tube(s) on ice or in a PCR tube chiller rack.

Component	Volume
Reaction volume from Step 3.8	20µl
DEPC Water	91µl
5X Second Strand Buffer	30µl
10mM dNTP Mix	3µl
10U/µl DNA Ligase	1µl
10U/µl DNA Polymerase I	4µl
2U/µl RNase H	1µl
Total	**150µl**

2. Mix gently (avoid vortexing). Briefly spin the tube(s) in a microcentrifuge. Incubate at 16°C for 2 hours.
3. Add 2µl of 5U/µl T4 DNA polymerase to each reaction. Incubate at 16°C for an additional 5 minutes. Do not allow the reaction temperature to exceed 16°C during this step.
4. **STOP POINT:** Place the sample(s) on ice or in a PCR tube chiller rack, and add 10µl of 0.5M EDTA. You can store samples overnight at -20°C.

Step 5. RNase A Cleanup

1. Add 1µl of 4mg/ml RNase A solution to the tubes from Step 4.4.

   Important: *Use caution when working with RNase A. Change gloves after use. Use RNase ZAP to clean work area surfaces.*

2. Mix gently (avoid vortexing). Briefly spin the tube(s) in a microcentrifuge.
3. Incubate sample(s) at 37°C for 10 minutes.
4. During incubation, centrifuge Phase Lock tube(s) at 12,000 x g for 2 minutes. Label one Phase Lock tube and two 1.5ml centrifuge tubes for each sample with sample names.
5. Add 163µl of phenol:chloroform:isoamyl alcohol to one set of 1.5ml centrifuge tubes.
6. Transfer the sample(s) to the tube(s) containing phenol:chloroform:isoamyl alcohol. Vortex well.
7. Transfer samples with the phenol:chloroform:isoamyl alcohol to Phase Lock tubes.
8. Centrifuge at 12,000 x g for 5 minutes.
9. Transfer the upper, aqueous layer to a clean 1.5ml tube.

Step 6. cDNA Precipitation

1. Add 16µl (0.1 volume of Step 5.9) of 7.5M ammonium acetate to the samples. Mix by repeated inversion. Briefly spin the tube(s) in a microcentrifuge.
2. Add 7µl of 5mg/ml glycogen to the samples. Mix by repeated inversion. Briefly spin the tube(s) in a microcentrifuge.
3. Add 326µl (2 volumes of Step 5.9) of ice-cold absolute ethanol to the samples. Mix by repeated inversion.
4. Centrifuge at 12,000 x g for 20 minutes.
5. Remove supernatant. Take care not to disturb the pellet.

6. Add 500µl of ice-cold 80% ethanol (v/v). Mix by repeated inversion.
7. Centrifuge tubes at 12,000 x g for 5 minutes.
8. Remove supernatant. Take care not to disturb the pellet.
9. Repeat steps 6 - 8.
10. Dry the pellet in a SpeedVac.
11. Rehydrate samples with 20µl of VWR water.

Step 7. Spectrophotometric QC of cDNA

1. Quantitate each cDNA sample according to the following formula:

   cDNA Concentration (µg/ml) = A_{260} x 50 x Dilution Factor

2. Verify that all samples meet the following requirements:

   - Concentration ≥ 100ng/µl
   - $A_{260}/A_{280} \geq 1.8$
   - $A_{260}/A_{230} \geq 1.8$

Step 8. Bioanalyzer/Gel QC of cDNA

1. Transfer 250ng cDNA to a sterile microcentrifuge tube. Store the remainder of your sample on ice or at -20°C.
2. Analyze the samples using the Agilent Bioanalyzer.
3. Compare the Bioanalyzer traces to the traces displayed below. Verify that all samples meet the following requirement for acceptance:

   - Median size ≥ 400bp when compared to a DNA ladder.
   - Looks similar to the examples of good cDNA sample traces displayed below.

   ***Note**: If using an agarose gel, compare the gel images to the Bioanalyzer's electropherogram images.*

   Samples exhibiting degradation should not be carried through labeling and hybridization because there is an unacceptable risk of poor results.

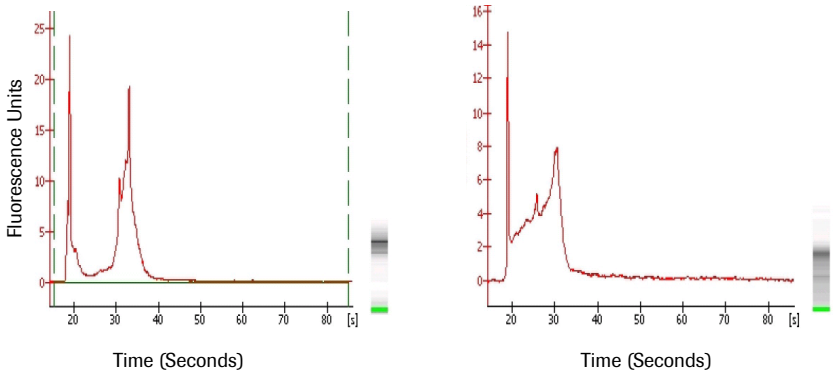


Figure 4: Example Traces Showing Good cDNA from a Eukaryotic Organism

Note: *Your traces could look different than these examples. Compare your traces to reference traces for the organism being researched.*

Chapter 3. Sample Labeling

Chapter 3 describes how to label your cDNA samples using a NimbleGen One-Color DNA Labeling Kit. Be aware of the following when using these kit contents:

- Aliquot dNTPs and Cy primer into single-use amounts.
- 5M NaCl could precipitate. Vortex if necessary.

1. Prepare the following solution:

Random Primer Buffer	All Array Formats	Notes
Random Primer Buffer (vial 2)	998.25µl	Prepare fresh buffer each time primers are resuspended.
β-Mercaptoethanol	1.75µl	
Total	**1ml**	

2. Briefly centrifuge Cy3-Random Nonamers (vial 3) because some of the product could have dislodged during shipping. Dilute the primer in 924µl of Random Primer Buffer with β-Mercaptoethanol. Aliquot to 40µl individual reaction volumes in 0.2ml thin-walled PCR tubes and store at -20°C, protected from light.

   Note: *Do not store diluted primers longer than 4 months.*

3. Assemble the following components in separate 0.2ml thin-walled PCR tubes:

Component	All Array Formats
cDNA	1µg
Diluted Cy3-Random Nonamers from step 2	40µl
Nuclease-free Water (vial 1)	To volume
Total	**80µl**

4. Heat-denature samples in a thermocycler at 98°C for 10 minutes. Quick-chill in an ice-water bath for 10 minutes.

Important: *Quick-chilling after denaturation is critical for high-efficiency labeling.*

5. Prepare the following dNTP/Klenow master mix for each sample prepared in step 4.

   Important: *Keep all reagents and dNTP/Klenow master mix on ice. Do not vortex after addition of Klenow.*

dNTP/Klenow Master Mix: Recipe per Sample	All Array Formats
10mM dNTP Mix (vial 5)	10µl
Nuclease-free Water (vial 1)	8µl
Klenow Fragment (3'->5' exo-) 50U/µl (vial 4)	2µl
Total	**20µl**

6. Add 20µl of the dNTP/Klenow master mix prepared in step 5 to each of the denatured samples prepared in step 4. Keep on ice.

Component	All Array Formats
Reaction volume from step 4	80µl
dNTP/Klenow Master Mix	20µl
Total	**100µl**

7. Mix well by pipetting up and down 10 times.

   Important: *Do not vortex after addition of Klenow.*

8. Quick-spin to collect contents in bottom of the tube.

9. Incubate at 37°C for 2 hours in a thermocycler with heated lid, protected from light.

10. Stop the reaction by addition of the Stop Solution (0.5M EDTA).

Component	All Array Formats
Reaction volume from step 6	100µl
Stop Solution (0.5M EDTA) (vial 6)	10µl
Total	**110µl**

11. Add 5M NaCl to each tube.

Component	All Array Formats
Reaction volume from step 10	110µl
5M NaCl (vial 7)	11.5µl
Total	**121.5µl**

12. Vortex briefly, spin, and transfer the entire contents to a 1.5ml tube containing isopropanol.

Component	All Array Formats
Reaction volume from step 11	121.5µl
Isopropanol	110µl
Total	**231.5µl**

13. Vortex well. Incubate for 10 minutes at room temperature, protected from light.
14. Centrifuge at 12,000 x g for 10 minutes. Remove supernatant with a pipette. Pellet should be pink.
15. Rinse pellet with 500µl 80% ice-cold ethanol. Dislodge pellet from tube wall by pipetting a few times.
16. Centrifuge at 12,000 x g for 2 minutes. Remove supernatant with a pipette.
17. Dry contents in a SpeedVac on low heat until dry (approximately 5 minutes), protected from light.
18. **STOP POINT:** Proceed to step 19, or store labeled samples at -20°C (up to 1 month), protected from light.
19. Spin tubes briefly prior to opening. Rehydrate pellets in 25µl Nuclease-free Water (vial 1) per reaction.
20. Vortex for 30 seconds and quick-spin to collect contents in bottom of the tube. Continue to vortex or let sit at room temperature, protected from light, for approximately 5 minutes or until the pellet is completely rehydrated, then vortex again and quick-spin.

21. Quantitate each sample using the following formula:

$$\text{Concentration } (\mu g/ml) = A_{260} \times 50 \times \text{Dilution Factor}$$

Note: *The NanoDrop user's manual specifies that in the Nucleic Acid module the maximum accurate reading is 3,700ng/µl, and in the Microarray module, the maximum accurate reading is 700ng/µl. Roche NimbleGen recommends using the spectrophotometer in the Nucleic Acid module. If sample concentration exceeds these values, dilute sample appropriately and requantitate.*

22. Based on the concentration, calculate the volume of Cy3-labeled cDNA sample required for each hybridization per the following table and aliquot in a 1.5ml tube:

Sample Requirements*	385K Array	4x72K Array	12x135K Array
Cy3-labeled cDNA Sample	Prok: 3µg Euk: 6µg	Prok: 2µg Euk: 4µg	Prok: 2µg Euk: 4µg

* Prok = Prokaryote; Euk = Eukaryote

23. Dry contents in a SpeedVac on low heat, protected from light.

24. **STOP POINT:** Proceed to Chapter 4, or store labeled samples at -20°C (up to 1 month), protected from light.

Chapter 4. Hybridization & Washing

Chapter 4 describes the NimbleGen protocol for sample hybridization and washing. Be aware of the following:

- The hybridization protocol requires a NimbleGen Hybridization System. Refer to its user's guide for specific instructions on its use.
- The hybridization protocol requires adhering a NimbleGen mixer to the microarray slide. Refer to the package label to identify the mixer design. Some instructions in the protocol are specific to the mixer design.
- The Alignment Oligo and Sample Tracking Controls (STCs) provided in the NimbleGen Hybridization and Sample Tracking Control Kits, respectively, are labeled with Cy dye, which are sensitive to photobleaching and freeze-thawing. After thawing stock tubes for the first time, aliquot the Alignment Oligo and STCs into single-use volumes and freeze at -20°C. Protect tubes from light.

Step 1. Prepare Samples

1. Set the Hybridization System to 42°C. With the cover closed, allow at least 3 hours for the temperature to stabilize.

   Note: *Be aware that the temperature of the Hybridization System may fluctuate during stabilization.*

2. Resuspend the dried sample pellet in VWR water (for 385K arrays) or appropriate Sample Tracking Control (for 4x72K and 12x135K arrays) according to the following table. Each sample to be hybridized to a 4x72K or 12x135K array should be resuspended in a unique STC. Record which STC is used for each sample.

Component	385K Array	Each Sample for a 4x72K Array	Each sample for a 12x135K Array
Reagent for resuspension	Water	Sample Tracking Control	Sample Tracking Control
Volume to add to Cy-labeled Sample from step 23 in Chapter 3	5µl	3.3µl	3.3µl

3. Vortex well and spin to collect contents in bottom of the tube.

4. Using components from a NimbleGen Hybridization Kit, prepare the hybridization solution master mix according to the following table. For 4x72K and 12x135K arrays, the amount listed is sufficient to hybridize all 4 or 12 subarrays on a slide. To hybridize multiple slides, adjust the amounts accordingly.

Hybridization Solution Master Mix to Hybridize a Single Slide	385K Array	4x72K Array	12x135K Array
2X Hybridization Buffer	11.8µl	29.5µl	88.5µl
Hybridization Component A	4.7µl	11.8µl	35.4µl
Alignment Oligo	0.5µl	1.2µl	3.6µl
Total	**17µl**	**42.5µl**	**127.5µl**

5. Add the appropriate amount of hybridization solution to each sample according to the following table:

Component	385K Array	Each Sample for a 4x72K Array	Each Sample for a 12x135K Array
Resuspended sample from step 2	5µl	3.3µl	3.3µl
Hybridization solution from step 4	13µl	8.7µl	8.7µl
Total	**18µl**	**12µl**	**12µl**

6. Vortex well (approximately 15 seconds) and spin to collect contents in bottom of the tube. Incubate at 95°C for 5 minutes, protected from light.

7. Place tubes at 42°C (in the Hybridization System sample block or heat block) for at least 5 minutes or until ready for sample loading. Vortex and spin prior to loading.

Step 2. Prepare Mixers

1. Locate the appropriate mixer. Remove from its package.

   Note: *For best results, use the NimbleGen Compressed Gas Nozzle to gently blow compressed inert gas across the mixer and slide to remove any dust or debris. The use of canned aerosol compressed air for this purpose is not recommended and could compromise array and data quality.*

   Load samples within 30 minutes of opening the vacuum-packaged mixer to prevent the formation of bubbles during loading and/or hybridization.

Array Format	Mixer
385K array	X1 mixer
4x72K array	X4 mixer
12x135K array	HX12 mixer

2. Position the Precision Mixer Alignment Tool (PMAT) with its hinge on the left. Open the PMAT (Figure 5).

3. Snap the mixer onto the two alignment pins on the lid of the PMAT with the tab end of the mixer toward the inside hinge and the mixer's adhesive gasket facing outward (Figure 5).

4. While pushing back the plastic spring with a thumb, place the slide in the base of the PMAT so that the barcode is on the right and the corner of the slide sits against the plastic spring. The NimbleGen logo and barcode number should be readable. Remove your thumb and make sure the spring is engaging the corner of the slide and the entire slide is registered to the edge of the PMAT to the rightmost and closest to you. In addition, be sure that the slide is lying flat against the PMAT. Gently blow compressed inert gas across the mixer and slide to remove dust.

   Note: *Take care to align the slide correctly in the PMAT. Incorrectly aligned slides may result in inaccurate attachment of the mixer and may not fit well into the Mixer Disassembly Tool used to remove the mixers after hybridization.*

5. Using forceps, remove the backing from the adhesive gasket of the mixer and close the lid of the PMAT so that the gasket makes contact with the slide.

6. Lift the lid by grasping the long edges of the PMAT while simultaneously applying pressure with a finger through the window in the lid of the PMAT to free the mixer-slide assembly from the alignment pins.

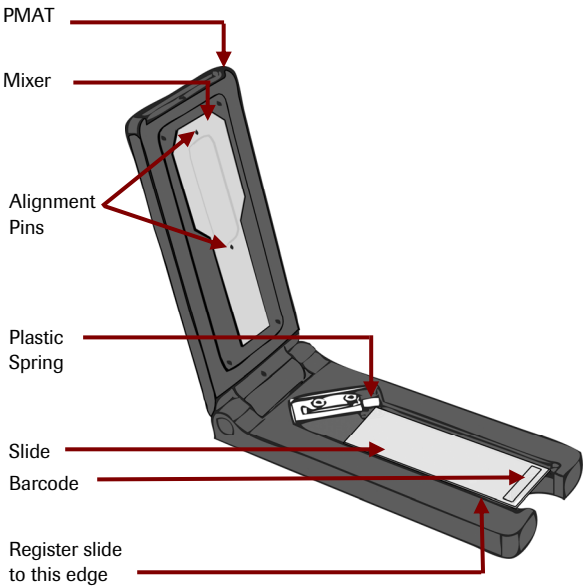


Figure 5: PMAT with X1 Mixer and Slide

7. Remove the mixer-slide assembly from the PMAT.
8. Place the mixer-slide assembly on the back of a 42°C heating block to facilitate adhesion of the mixer to the slide.
9. Rub the Mixer Brayer over the mixer with moderate pressure to adhere the adhesive gasket and remove any bubbles. For X1 mixers, start in the center of the array and rub outwards. For X4 and HX12 mixers, first use a corner of the Mixer Brayer to rub the borders between the subarrays, and then rub around the outside of the subarrays. The adhesive gasket will become clear when fully adhered to both surfaces.
10. Place the mixer-slide assembly in the slide bay of the Hybridization System.

Step 3. Load & Hybridize Samples

1. Refer to the appropriate diagram below when loading sample:

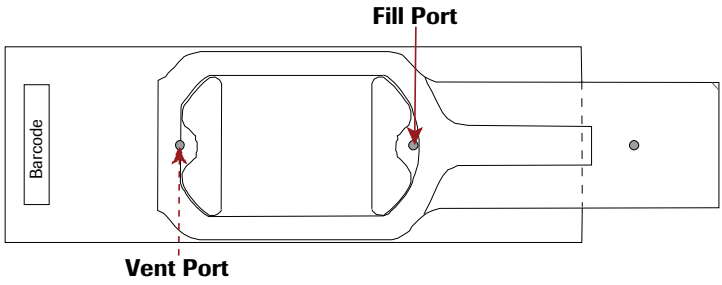


Figure 6: X1 Mixer and Slide for a 385K Array

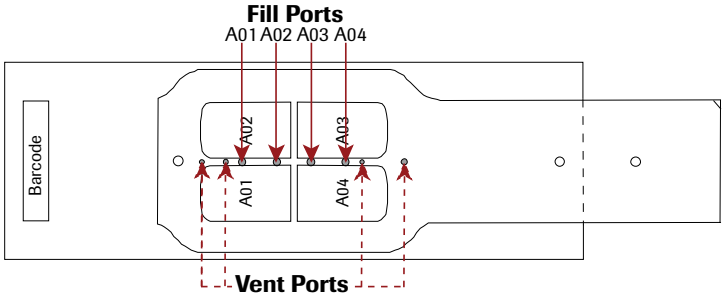


Figure 7: X4 Mixer and Slide for a 4x72K Array

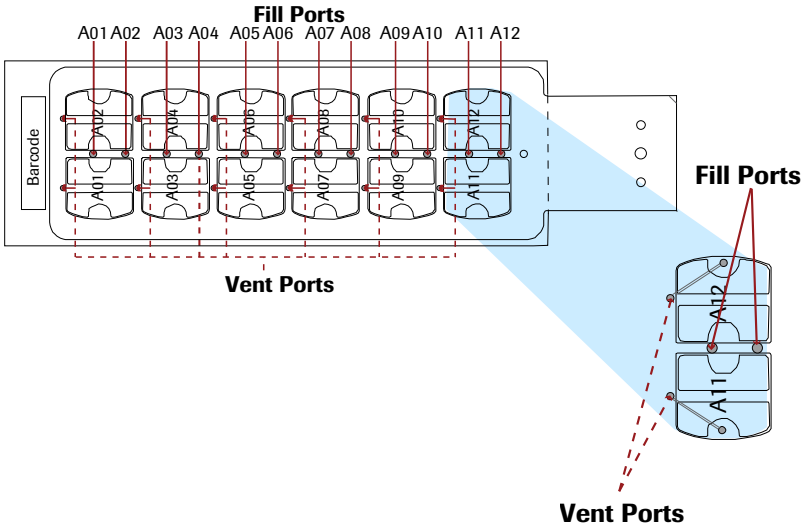


Figure 8: HX12 Mixer for a 12x135K Array. The enlarged image shows the positioning of fill and vent ports on the HX12 mixer.

2. Keep the following in mind before loading sample:

- Leave residual volume in the sample tube to avoid bubbles. The volumes listed in the table below account for this additional amount.
- After aspirating the designated sample volume, inspect the pipette tip for air bubbles. Dispense and reload the pipette if bubbles exist.

Keep the following in mind when loading sample:

- Keep the pipette tip perpendicular to the slide to avoid possible leakage at the fill port.
- Apply gentle pressure of the tip into the port to ensure a tight seal while loading the sample.

Component	385K Array	4x72K Array	12x135K Array
Sample Loading Volume	16µl	8µl	6µl
Pipette Tip	CP100	CP10*	CP10*

* The CP10 tip is thin and flexible. Place the thumb and forefinger of your free hand on the tip to guide it into the port.

3. Using the appropriate Gilson Microman pipette, slowly dispense the appropriate sample volume into the fill port. Load samples and seal mixer ports as described below for each array format:

   - For 385K arrays:

     a. Load sample into the fill port. Dry any overflow from the fill and vent ports with a cotton swab after loading the array.

     b. Use one mixer port seal to cover the fill port and another to cover the vent port on X1 mixer. Press the mixer port seal using uniform pressure across the seal to adhere.

     c. Use forceps to press the mixer port seal around the fill and vent ports to ensure it is adhered in those areas.

   - For 4x72K arrays:

     a. Load sample into the A01 fill port. Dry any overflow from the fill and vent ports with a cotton swab after loading. Repeat loading samples into the A02 - A04 fill ports, using a fresh cotton swab for drying the ports for each array.

     b. Use one mixer multi-port seal to cover all fill and vent ports on X4 mixers. Press the mixer multi-port seal, using uniform pressure across the seal to adhere.

     c. Use forceps to press the mixer multi-port seal around the fill and vent ports to ensure it is adhered in those areas.

   - For 12x135K arrays:

     a. Load sample into the A01 fill port. Due to the close proximity of the fill and vent ports, do not overfill the arrays. Load sample until it enters the vent port channel. Do not allow sample to come to the surface of the HX12 mixer. If overflow occurs, dry the overflow from the fill and vent ports with a cotton swab after loading. Repeat loading sample into the A02 - A06 fill ports, using a fresh cotton swab for drying the ports for each array.

     b. Use one mixer multi-port seal to cover A01 - A06 fill ports. Apply light pressure at each fill port to seal.

     c. Use one mixer multi-port seal to cover A01, A03, A05 vent ports and one mixer multi-port seal to cover A02, A04, A06 vent ports. Apply light pressure at each vent port to seal.

     d. Repeat steps a - c to load samples into A07 - A12.

4. Close the bay clamp.
5. Turn on the Mixing Panel on the Hybridization System, set the mix mode to B, and press the mix button to start mixing. Confirm that the Hybridization System recognizes the slide in each occupied bay (its indicator light becomes green).
6. Approximately 10 minutes after starting the Hybridization System:
   - Ensure the mix mode is set to B.
   - Ensure a green light is displayed for all occupied stations.
7. Hybridize sample at 42°C to the array(s) for 16 - 20 hours.

Step 4. Wash Hybridized Arrays

Important: *To ensure high quality data, it is important to proceed through all the washing and drying steps without interruption. The NimbleGen Microarray Dryer dries up to 24 slides at a time. If using a microarray dryer that dries one slide at a time, wash only one slide at a time.*

1. Locate the components of the NimbleGen Wash Buffer Kit and NimbleGen Array Processing Accessories (refer to page 7).
2. Before removing the mixer-slide assemblies from the Hybridization System, prepare Washes I, II, and III according to the following tables. Note that you prepare two containers of Wash I.

Washing Multiple Slides	Wash I (user-supplied dish[1])	Washes I, II, and III (wash tank[2])
VWR Water	225ml	225ml
10X Wash Buffer I, II, or III	25ml	25ml
1M DTT	25µl	25µl
Total	**250ml**	**250ml**
Washing One Slide	**Wash I (user-supplied dish[1])**	**Washes I, II, and III (slide container[2])**
VWR Water	225ml	22.5ml
10X Wash Buffer I, II, or III	25ml	2.5ml
1M DTT	25µl	2.5µl
Total	**250ml**	**25ml**

1 Ensure that this dish is shallow and wide enough to accommodate the mixer-slide assembly loaded in the Mixer Disassembly Tool. This dish must also be small enough to ensure that the Mixer Disassembly Tool is completely submerged in the wash solution.

2 If washing multiple slides, prepare the washes in the wash tanks. If washing only one slide, prepare the washes in the slide containers.

3. To facilitate the removal of the mixer, heat the shallow dish containing Wash I to 42°C. Roche NimbleGen recommends measuring the temperature of Wash I at every use. Keep the remaining three wash solutions at room temperature.

4. Insert the Mixer Disassembly Tool into the shallow dish containing warm Wash I. If you will be washing multiple slides, insert a slide rack into the wash tank containing Wash I at room temperature.

5. Remove a mixer-slide assembly from the Hybridization System and load it into the Mixer Disassembly Tool immersed in the shallow dish containing warm Wash I.

   Important: *Do not allow the mixer-slide assembly to cool before removing the mixer. Keep power on to the Hybridization System's heat block and mixer system during mixer-slide disassembly, and transfer each mixer-slide assembly one at a time to Wash I for immediate removal of the mixer.*

6. With the mixer-slide assembly submerged, carefully peel the mixer off the slide. It is important to hold the Mixer Disassembly Tool flat while removing the mixer and to avoid any horizontal movement or scraping with the mixer across the slide. Do not touch the array surface of the slide.

   ***Important**: The mixer is extremely flexible. Peel the mixer off slowly to avoid breaking the slide.*

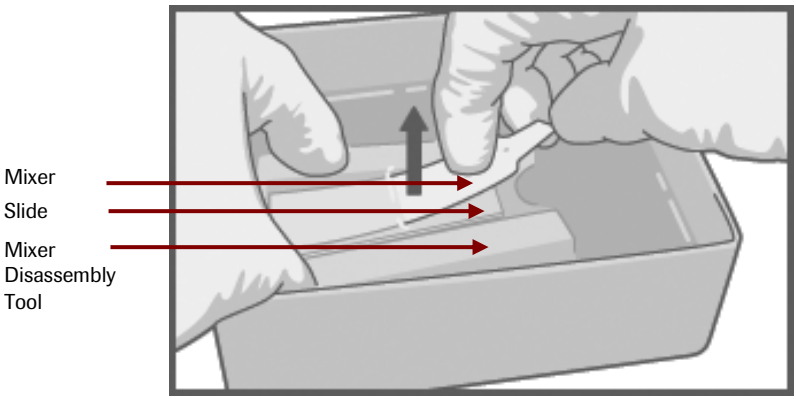


Figure 9: Using the Mixer Disassembly Tool to Remove a Slide from a Mixer

7. Working quickly, discard the mixer and remove the slide from the Mixer Disassembly Tool.

8. Gently agitate the slide for 10 - 15 seconds in the shallow dish containing warm Wash I to quickly remove the hybridization buffer.

   ***Note**: It is important for achieving good array uniformity to quickly and evenly wash the hybridization buffer off the slide surface as soon as the mixer is removed.*

9. If washing multiple slides, transfer the slide with the barcode at the top into a slide rack in the wash tank that contains Wash I. If washing one slide, transfer the slide into a slide container that contains Wash I. Agitate vigorously for 10 - 15 seconds.

   Slide rack users: To ensure high quality data, make sure the microarray area of the slide remains submerged at all times during all wash steps.

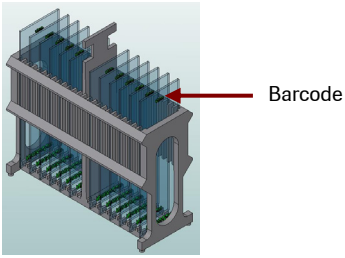


Figure 10: Insert Slides with the Barcode at the Top into the Slide Rack

Note: If you are using a NimbleGen Microarray Dryer or other microarray dryer that dries multiple slides at a time, repeat steps 4 - 9 until you have removed the mixer from all slides to wash. Load each slide into the slide rack with the array facing the same direction.

10. Wash for an additional 2 minutes in Wash I with vigorous, constant agitation. If washing multiple slides, move the rack up and down with enough agitation to make foam appear. If washing one slide, shake the slide container at least 20 times every 10 seconds.

    ***Important**: At several times during the wash, rock the wash tank so the wash solution covers and cleans the top of the slide(s).*

11. Quickly blot the rack, or edges of the slide if only washing one slide, several times using paper towels to remove excessive buffer carryover. Transfer the slide(s) to Wash II and wash for 1 minute with vigorous, constant agitation. If washing multiple slides, rock the wash tank so the wash solution covers and cleans the tops of the slide(s).

    ***Important:** Do not allow slides to dry between wash steps.*

12. Transfer the slide(s) to Wash III and wash for 15 seconds with vigorous, constant agitation. If washing multiple slides using the slide rack, rock the wash tank so the wash solution covers and cleans the tops of the slide(s).

13. Remove the slide(s) from Wash III. Spin dry in a NimbleGen Microarray Dryer or other microarray dryer per the manufacturer's recommendation. For a NimbleGen Microarray Dryer, the recommended drying time is 2 minutes (120 seconds).

14. Remove the slide(s) from the NimbleGen Microarray Dryer or other microarray dryer. Blot dry the edges to remove any residual moisture.

    ***Note**: When not in use, store the dried slide in its original slide case in a dark desiccator.*

15. Proceed immediately to the steps for scanning the array(s) in Chapter 5.

Notes

Chapter 5. One-Color Array Scanning

Chapter 5 describes the protocol for scanning one-color NimbleGen arrays with a GenePix 4000B Scanner and associated software. Keep arrays in a dark desiccator until you are ready to scan them.

1. Turn on the scanner using the power switch on the back right side.
2. Launch the GenePix software 10 minutes before scanning to allow lasers to warm.
3. Open the scanner door and the slide carriage.
4. Using a NimbleGen Compressed Gas Nozzle, gently blow compressed inert gas across the slide to remove any dust or debris from the array. Do not use canned aerosol compressed air for this purpose.
5. Place the slide in the slide carriage so that the array is face down and the barcode end is closest to you.
6. Move the black lever on the left side of the carriage to the left until the slide is lying flat in the carriage.
7. Release the lever so that the slide is gently pushed to the right side of the carriage and held firmly.
8. Close the carriage (you should hear a click) and slide the scanner door shut.
9.
Open the Hardware Settings dialog box (right side, second icon from the bottom or Alt + Q). Select the following settings for scanning:
    - Wavelength = 532 (Cy3)
    - PMT Gain = 500
    - Power (%) = 100
    - Pixel size (µm) = 5
    - Lines to average = 1
    - Focus position (µm) = 0

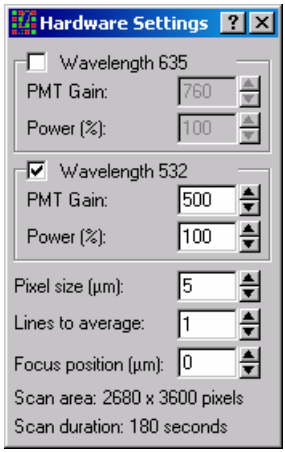


Figure 11: Hardware Settings Dialog Box

10. Under **Image** on the left side of the screen, select the 532 wavelength.
11.
Preview the entire slide by clicking the preview scan icon (or Alt + P). This will allow you to locate the array(s) on the slide.

12. Under **Tools** on the left side of the screen, click the scan area icon (or Ctrl + V). Click and drag to define a box that surrounds the array image on 385K arrays, all 4 subarray images on 4x72K arrays, or all 12 subarray images on 12x135K arrays. The box dimensions should be stretched to create a box just slightly larger than the array image(s). Balance the border around the scanning area to ensure proper data extraction later. It is critical to include all of the corner probes within the scan image.

Note: *Only one scan is required per slide. It is not necessary to scan each subarray individually. Be sure to scan the entire slide regardless of whether each subarray was used.*

Approximate coordinates and size of the scan area are as follows and can be entered into the Scan Area Properties (F10):

Coordinate	385K and 4x72K Array	12x135K Array
Left	4,200	2,600
Top	26,000	2,800
Width	13,400	15,500
Height	18,000	62,200

13. Scan the array by clicking the play icon (or Alt + D). While scanning, set the zoom to view the whole image and adjust the brightness and contrast of the displayed image to eliminate visible saturation.

Switch to zoom mode by clicking on the array image and pressing Z on the keyboard. Click and drag the cursor over the region to zoom. To restore the prior view, press Alt + Z. Center the entire array image in the viewing window.

14. Zoom into a region scanned under the most recent PMT gain. Zoom into as large a region as possible to get an accurate reading for the whole array. If there is high background outside of the array area, avoid these regions. The histogram is calculated from all regions bound by the current view, but ratios must be selected for array areas only.

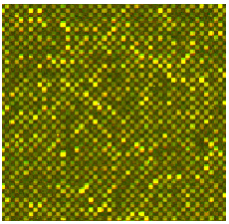


Figure 12: Example Array Features

- On the top left side of the screen, make sure the *Wavelength 532* box is checked.
- On the left side of the screen, under *Y-Axis*, make sure the *Log Axis* box is checked.

- You want the curve to have 1e-5 normalized counts at the 65,000-intensity level (saturation).
    - If the normalized counts at the 65,000-intensity level are less than 1e-5, increase the PMT gain (Hardware Settings dialog box).
    - If the normalized counts at the 65,000-intensity level are more than 1e-5, decrease the PMT gain (Hardware Settings dialog box).
    - The histogram graphs the region of the image viewable on-screen in the image tab. If the histogram is no longer changing, either the scan is complete or area on the current view has been scanned.

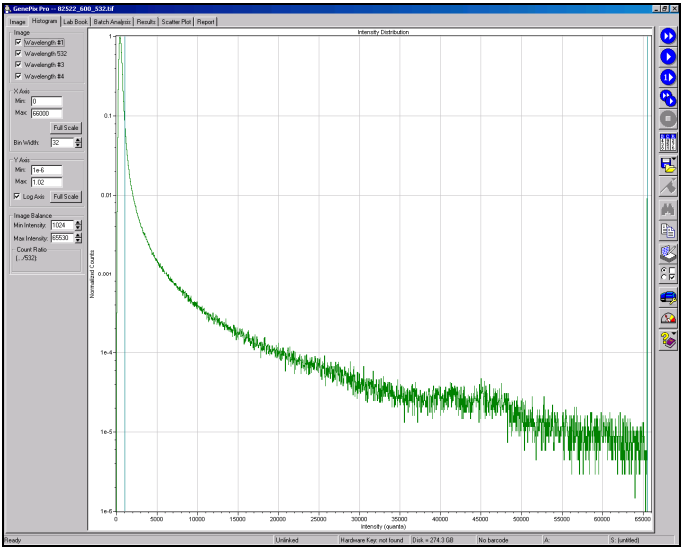


Figure 13: Example Histogram

15. After the PMT gain is properly adjusted, stop then restart the scan under the new setting.

16. When the scan completes, zoom into each corner and confirm all features are visible. If some features are missing, refer to step 12. Adjust the scanning area appropriately and rescan.

17. Save the images using the following naming convention:

    NNNNN_XXXXX_532.tif

    where **NNNNN** is the slide's barcode number and **XXXXX** is optional user-defined text.

18. (For 4x72K or 12x135K arrays only) If the signal intensities between the subarrays are not uniform, you can scan the slide multiple times, each time adjusting the PMT gain to acquire the best image of a specific subarray. Alternatively, scan and save each subarray individually (as described in the following section).

    Scan and save the images as described in steps 17 - 19, specifying the subarray for which the PMT gain was optimized in the file name.

19. After scanning, remove the slide from the scanner. Store slides in a dark desiccator in the event that rescanning is necessary.

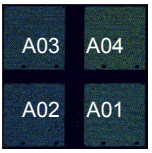
Scanning Individual Subarrays on Multiplex Arrays

If the signal intensities between subarrays on 4x72K or 12x135K (multiplex) arrays are not uniform, you can scan individual subarray(s) with a higher or lower PMT gain, as appropriate, to acquire the best image of the subarray(s). The locations of individual subarrays on the scanned image are shown in Figure 14. The coordinates in the associated tables may be used for defining the scan area for an individual subarray.

Scan and save the image as described in steps 15 - 17 above, specifying the subarray and the PMT gain in the file name.

***Important**: When viewing this scanned image in NimbleScan software, you do not need to burst (separate) its file as described in Chapter 6.*

4x72K Array	Left	Top	Width	Height
A01	11,540	36,075	6,100	8,340
A02	4,100	36,075	6,100	8,340
A03	4,100	26,420	6,100	8,340
A04	11,540	26,420	6,100	8,340



12x135K Array	Left	Top	Width	Height
A01	11,250	54,950	6,850	9,200
A02	3,175	54,950	6,850	9,200
A03	11,250	44,500	6,850	9,200
A04	3,175	44,500	6,850	9,200
A05	11,250	33,700	6,850	9,200
A06	3,175	33,700	6,850	9,200
A07	11,250	23,300	6,850	9,200
A08	3,175	23,300	6,850	9,200
A09	11,250	12,600	6,850	9,200
A10	3,175	12,600	6,850	9,200
A11	11,250	2,150	6,850	9,200
A12	3,175	2,150	6,850	9,200

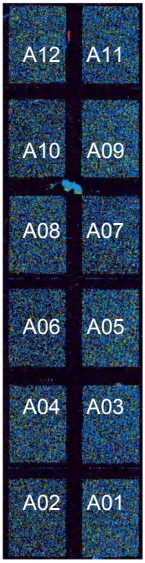


Figure 14. Dimensions and Example Images of Multiplex Arrays

Notes

Chapter 6. NimbleScan Data Analysis

Chapter 6 describes how to import your scanned image and extract the data using Roche NimbleScan software. Refer to the *NimbleScan v2.5 User's Guide* for computer system requirements and detailed information on using the software.

Step 1. Burst Multiplex Image (4x72K and 12x135K Arrays Only)

If your files contain the scanned images of 4x72K or 12x135 arrays, follow the steps below to burst (separate) each slide image into 4 or 12 separate array images, respectively. Otherwise, proceed to "Step 2. Import Image."

1. Select **File** -> **Burst Multiplex Image**. The Burst Multiplex Image dialog box appears.
2. To choose the .tif files to burst, click **Add images**.
3. Navigate to the directory containing your array image file (.tif) and select the file.
4. Click **Add to batch**. Multiple slide images can be burst at once.
5. Browse to select the correct multiplex description file (.ncd) in the Design File disk provided with the 4x72K or 12x135K array for the *Multiplex description file* field.
6. Browse to select the desired output file destination in the *Output burst images to* field.
7. Click **Burst**. NimbleScan software creates 4 image files from a 4x72K array image or 12 image files from a 12x135K array image. The subarray designation (A01, A02, etc.) for each bursted image is specified in the original image's file name. For example:
   - **NNNNN**A01_XXXXX_532.tif…**NNNNN**A04_XXXXX_532.tif
   - **NNNNN**A01_XXXXX_532.tif…**NNNNN**A12_XXXXX_532.tif

   where **NNNNN** is the slide's barcode number and **XXXXX** is optional user-defined text.
8. Repeat steps 1 - 7 for each multiplex image.

   Note: *Alternatively, you can burst all images designed with the same layout file in a batch mode by selecting all files to burst in step 2 above.*

Step 2. Import Image

1. Select **File** -> **Open**. The Open an Alignment dialog box appears.
2. Navigate to the directory containing your array images and select one image. If you scanned multiplex arrays at multiple PMT gain settings, remember to select the optimized image of each array for further analysis.
3. Select the design file (.ndf) in the Design File disk provided with your array. This file describes the placement of the probes on the array.
4. Select the gene description file (.ngd) in the Design File disk provided with your array. This file contains NimbleGen sequence identifiers as well as information about the sequence.
5. Click **Open**.

Step 3. Extract Image

1. Select the auto brightness/contrast adjust function.

2. Select the auto align tool to overlay the grid on the array.

Note: *If the software indicates auto alignment was not successful, perform a manual alignment as described in the NimbleScan v2.5 User's Guide.*

3. Zoom into the top corner of the array with the zoom tool.

Check to make sure all fiducial controls align correctly with the grid.

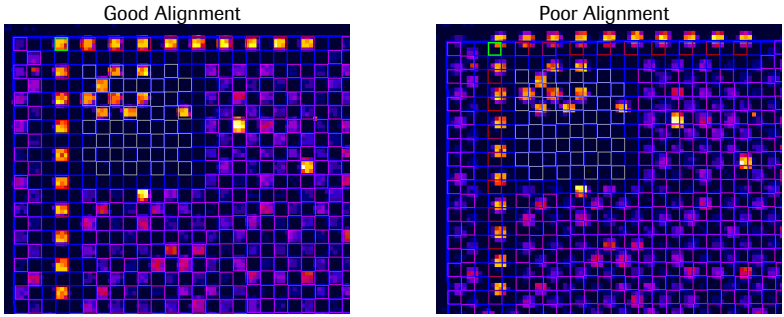


Figure 15: Examples Showing Good and Poor Grid Alignments

4. If necessary, adjust the grid by clicking on the green highlighted square located in the corner. Move this square so that the corner fiducial controls line up correctly with the grid. Move to each corner using the jump to location buttons. For 385K arrays, also check the center fiducial controls.

5. Select the local alignment tool to fine-tune the alignment.

6. Click **File** -> **Save** to save the gridded image.

7. If you are analyzing data from a 4x72K or 12x135K array, proceed to "Step 4. Confirm Experimental Integrity (4x72K and 12x135K Arrays Only)." If you are analyzing data from a 385K array, proceed directly to "Step 5. Generate an Experimental Metrics Report."

Step 4. Confirm Experimental Integrity (4x72K and 12x135K Arrays Only)

A unique STC should have been added to each sample prior to loading onto 4x72K and 12x135K arrays as described in Chapter 4, Step 1. This control hybridizes to probes on the microarray and enables you to confirm the sample identity on each array and ensure integrity of the experiment. STC probes are placed as repeating sets of 20 along the perimeter of each array and as two 4 x 5 blocks in the upper left corner and in the center of the array (Figure 16). Roche NimbleGen recommends performing a sample tracking analysis (refer to page 45) and visually checking the STC features along the perimeter (refer to page 47) to confirm that the correct sample has been added to each array.

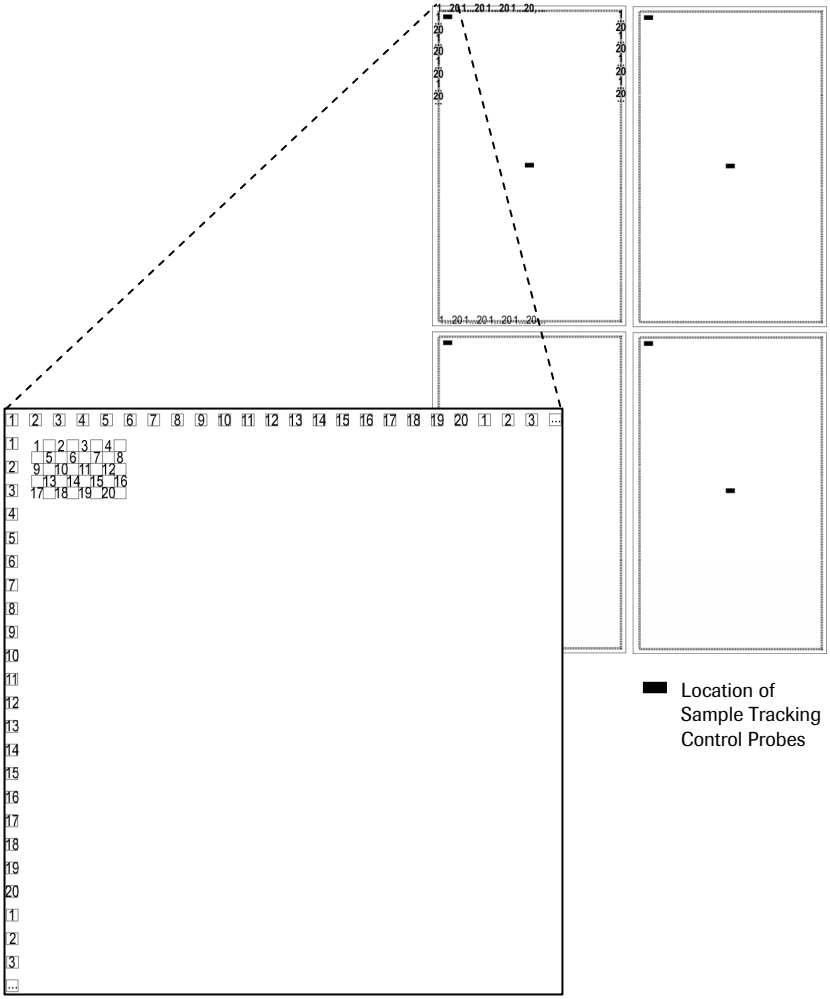


Figure 16: Location and Numbering of Sample Tracking Control Probes on a 4x72K Array

Performing a Sample Tracking Analysis

When you run a Sample Tracking analysis, NimbleScan software generates a Sample Tracking report based on signal intensities of features in the two 4 x 5 blocks (Figure 16). Using this report and sample records from your experiment, you can confirm the intended sample was hybridized to the intended array.

1. Select **Analysis** -> **Sample Tracking**.
2. Click **Add files**.
3. Select all gridded images for the experiment (for example, select 4 images for 4x72K arrays).
4. Click **Add to List**.
5. Click **Browse** to select the destination of the output file. To view the output file in Microsoft Excel®, save as an .xls file.
6. Click **Run** to start the analysis.
7. Open the Sample Tracking report in spreadsheet software, such as Microsoft Excel. An example report is shown in Figure 17.
    a. Confirm that the Image ID, Design ID, and Design Name are accurate.
    b. Enter the Sample Name and Input STC in the appropriate spreadsheet cells. The Input STC is the unique STC number added to each sample before hybridization.
    c. For each array, review the signal intensity and the Absent or Present call for each of the up to 20 STCs that Roche NimbleGen may provide. The STC identified as Present should be the same as the Input STC you entered for the sample.
    d. Ensure that a Present call is reported for only the unique STC added to the sample. If more than one Present call is reported, the integrity of your data could be compromised due to cross-contamination that occurred during sample preparation, loading, or hybridization. The extent of cross-contamination that can be tolerated depends on your samples, experimental setup, and experimental goals. Roche NimbleGen recommends repeating experiments that show cross-contamination.
    e. Select **File** -> **Save** to save the changes to the file.

Image ID	109037A01_510_532	109037A02_510_532	109037A03_510_532	109037A04_510_532
Design ID	5010	5010	5010	5010
Design Name	HG18_60mer_expr	HG18_60mer_expr	HG18_60mer_expr	HG18_60mer_expr
Sample Name				
Array Number	A01	A02	A03	A04
Input STC				
STC 1 Intensity	39528	210	210	218
STC 2 Intensity	215	228	253	207
STC 3 Intensity	182	48920	232	206
STC 4 Intensity	191	214	221	250
STC 5 Intensity	195	248	40937	244
STC 6 Intensity	183	242	193	216
STC 7 Intensity	200	252	212	36140
STC 8 Intensity	202	198	212	187
STC 9 Intensity	186	213	208	199
STC 10 Intensity	223	240	203	214
STC 11 Intensity	226	215	216	237
STC 12 Intensity	171	240	185	207
STC 13 Intensity	199	225	206	209
STC 14 Intensity	176	217	264	223
STC 15 Intensity	207	240	233	207
STC 16 Intensity	168	242	206	195
STC 17 Intensity	239	252	268	258
STC 18 Intensity	225	227	212	207
STC 19 Intensity	211	226	219	221
STC 20 Intensity	259	239	228	221
STC 1 Call	Present	Absent	Absent	Absent
STC 2 Call	Absent	Absent	Absent	Absent
STC 3 Call	Absent	Present	Absent	Absent
STC 4 Call	Absent	Absent	Absent	Absent
STC 5 Call	Absent	Absent	Present	Absent
STC 6 Call	Absent	Absent	Absent	Absent
STC 7 Call	Absent	Absent	Absent	Present
STC 8 Call	Absent	Absent	Absent	Absent
STC 9 Call	Absent	Absent	Absent	Absent
STC 10 Call	Absent	Absent	Absent	Absent
STC 11 Call	Absent	Absent	Absent	Absent
STC 12 Call	Absent	Absent	Absent	Absent
STC 13 Call	Absent	Absent	Absent	Absent
STC 14 Call	Absent	Absent	Absent	Absent
STC 15 Call	Absent	Absent	Absent	Absent
STC 16 Call	Absent	Absent	Absent	Absent
STC 17 Call	Absent	Absent	Absent	Absent
STC 18 Call	Absent	Absent	Absent	Absent
STC 19 Call	Absent	Absent	Absent	Absent
STC 20 Call	Absent	Absent	Absent	Absent

Figure 17: Example of a Sample Tracking Report

Visually Checking STC Features

1. If necessary, zoom into the upper left corner of the array with the zoom tool.

2. Locate the repeating set of 20 features along the perimeter of the array. Figure 18 shows an example of an array hybridized with a sample containing STC 5.

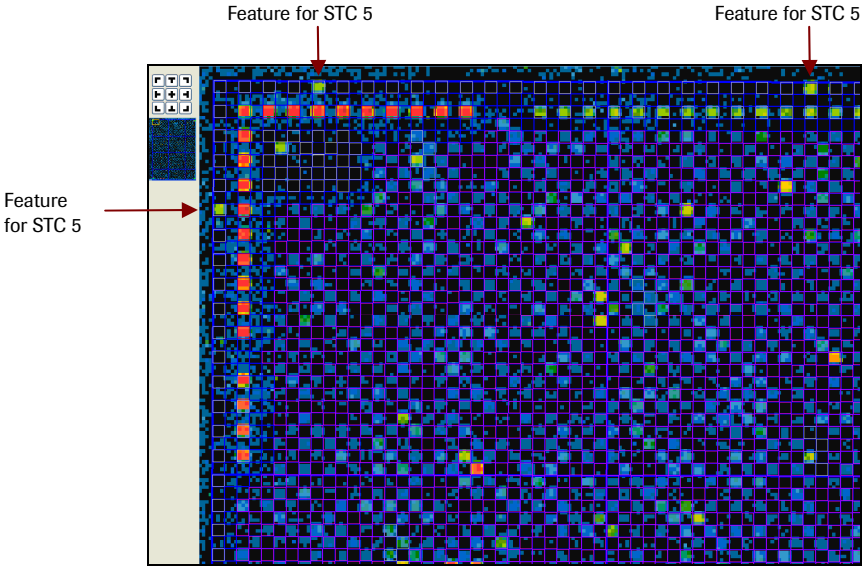


Figure 18: Example of an Array Hybridized with Sample Containing STC 5

3. Survey the entire perimeter of the array. If more than one STC is visible, the integrity of your data could be compromised due to cross-contamination that occurred during sample preparation, loading, or hybridization. Roche NimbleGen recommends repeating experiments that show cross-contamination.

Step 5. Generate an Experimental Metrics Report

The Experimental Metrics report provides a set of metrics that can be used to establish guidelines for determining the quality of the data. Roche NimbleGen recommends that you use these metrics to develop criteria for assessing the overall quality of your microarray experiments. The metrics provided will vary according to application, array format, probe design, organism, sample type and quality, and hybridization conditions. Refer to the "Guide to Interpreting the Experimental Metrics Report" provided with NimbleScan v2.5 software.

1. Select **Analysis -> Generate Experimental Metrics Report**.

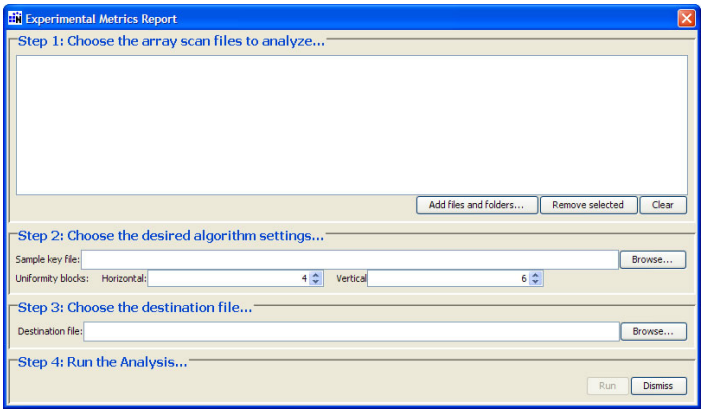


Figure 19: Experimental Metrics Report Dialog Box

2. In *Step 1: Choose the array scan files to analyze…*:
   - Click **Add files and folders** to select all gridded images (.tif) for the experiment (for example, select 12 images for 12x135K arrays). When the Open dialog box opens, navigate to the location of the files. You can select multiple files by pressing the Ctrl key and clicking each file name.
   - If you choose the wrong file, click the file name in the list box, and click **Remove selected**.
   - To remove all selected files, click **Clear**.

3. In *Step 2: Choose the desired algorithm settings…*:
   - (Optional) Click **Browse** to select the sample key file.
   - (Optional) Adjust the settings in the *Uniformity blocks* fields. For 2.1M arrays, Roche NimbleGen recommends the following settings: 4 for horizontal and 18 for vertical. For all other array formats (385K, 4x72K, etc.), we recommend the default settings (4 for horizontal and 6 for vertical).
4. In *Step 3: Choose the destination file…*, type the path to the folder in the *Destination file* field or click **Browse** to open a dialog box for navigating to the destination folder.
5. In *Step 4: Run the Analysis…*, click **Run** to start the analysis.
6. Open the Experimental Metrics report in spreadsheet software, such as Microsoft Excel. An example report is shown in Figure 20.

IMAGE_NAME	INTER QUARTILE_DENSITY	RATIO_RANGE	SIGNAL_RANGE	UNIFORMITY_MEAN	UNIFORMITY_CV	NUM_EMPTY	MEAN_EMPTY	NUM_EXPERIMENTAL	MEAN_EXPERIMENTAL	NUM_RANDOM	MEAN_RANDOM
10464202_532.tif	3.929	0	0.266	3306.222	0.07	394459	230.215	388502	3544.292	7500	153.769
10468702_532.tif	3.003	0	0.311	2700.208	0.104	394459	241.554	388502	2847.885	7500	217.502
11019502_532.tif	4.268	0	0.197	3561.511	0.052	394459	181.37	388502	3582.704	7500	129.186
11513902_532.tif	3.396	0	0.365	2888.551	0.078	394459	308.774	388502	2904.282	7500	204.956
12280602_532.tif	3.013	0	0.247	3200.017	0.064	394459	236.738	388502	3212.244	7500	228.447
12280802_532.tif	2.681	0	0.213	3015.984	0.048	394459	178.734	388502	3024.112	7500	293.124
12281502_532.tif	2.813	0	0.154	2921.114	0.043	394459	320.085	388502	2997.033	7500	273.547
12281602_532.tif	2.395	0	0.233	2696.795	0.052	394459	365.028	388502	2704.292	7500	354.416
12283702_532.tif	2.225	0	0.218	2622.463	0.049	394459	261.77	388502	2673.215	7500	277.471
12283902_532.tif	3.385	0	0.233	3183.686	0.053	394459	239.086	388502	3259.771	7500	189.811
12287002_532.tif	3.012	0	0.156	2809.022	0.032	394459	183.669	388502	2838.363	7500	196.522
12288902_532.tif	3.237	0	0.229	2672.599	0.055	394459	230.96	388502	2690.111	7500	177.77
12291702_532.tif	2.629	0	0.231	2978.218	0.051	394459	222.683	388502	2987.358	7500	289.519
12620402_532.tif	1.86	0	0.37	2356.775	0.095	394459	259.3	388502	2405.375	7500	368.063
12621302_532.tif	1.902	0	0.215	2471.146	0.056	394459	223.332	388502	2488.388	7500	399.323
12624502_532.tif	2.253	0	0.335	2314.496	0.071	394459	237.858	388502	2362.219	7500	229.138
12630402_532.tif	2.328	0	0.19	2465.049	0.044	394459	251.941	388502	2472.567	7500	246.527
12644902_532.tif	2.195	0	0.305	2833.377	0.069	394459	248.539	388502	2850.29	7500	298.537
12645002_532.tif	2.303	0	0.275	2589.061	0.07	394459	186.707	388502	2631.896	7500	213.644
12646702_532.tif	2.272	0	0.444	2319.074	0.123	394459	246.438	388502	2327.482	7500	255.542
12648802_532.tif	2.28	0	0.28	2933.423	0.07	394459	219.698	388502	2936.663	7500	279.281
12650902_532.tif	2.114	0	0.226	2120.828	0.056	394459	261.275	388502	2165.454	7500	258.655
12652002_532.tif	2.374	0	0.311	2338.651	0.078	394459	231.153	388502	2409.161	7500	232.67
12726002_532.tif	1.873	0	0.465	2444.016	0.112	394459	328.27	388502	2481.516	7500	361.771
12852502_532.tif	2.575	0	0.187	2666.4	0.063	394459	396.124	388502	2673.684	7500	255.828
13300102_532.tif	2.421	0	0.191	2580.928	0.055	394459	207.548	388502	2657.978	7500	240.56

Figure 20: Example of an Experimental Metrics Report

Following is a description of the contents of the Experimental Metrics report:

- *IMAGE_NAME*. The name of the analyzed image file.
- *INTERQUARTILE_DENSITY*. The interquartile range (IQR) of the raw signal intensities.
- *RATIO_RANGE*. The ratio range for two-color microarray applications. Ratio range is calculated by dividing the array into a grid of uniformity blocks (4 x 18 on 2.1M arrays and 4 x 6 on 385K, 4x72K, or 12x135K arrays), calculating the average $\log_2$ ratio of each block and calculating the range from the block averages.

  Note: *This metric applies only to two-color applications.*
- *SIGNAL_RANGE*. The signal range represents the signal range on a per channel basis for both one- and two-color microarray applications. Similar to ratio range, the signal range is calculated based on the range of signal means from the average signal per block.
- *UNIFORMITY_MEAN*. The mean signal intensity of all the probes in each uniformity block.
- *UNIFORMITY_CV*. The coefficient of variation of the block uniformity means.
- *NUM_EMPTY*. The number of empty features present on the array.
- *MEAN_EMPTY*. The mean signal intensity of empty features present on the array.
- *NUM_EXPERIMENTAL*. The number of experimental features present on the array.
- *MEAN_EXPERIMENTAL*. The mean signal intensity of the experimental features present on the array.
- *NUM_RANDOM*. The number of random control features present on the array.
- *MEAN_RANDOM*. The mean signal intensity of the random control features present on the array. These probes generally have the same length and GC characteristics as the experimental probes on the array, and can be used to estimate the amount of non-specific binding in the hybridization.

Step 6. Create Pair Reports

Pair reports contain the raw data, listing the probe intensities of the array. Create .pair files for all arrays to be analyzed together.

1. Select **Analysis** -> **Reports** -> **Pair**. Refer to the *NimbleScan v2.5 User's Guide* for further details on Pair reports.
2. To choose the gridded files to analyze, click **Add images**.
3. Navigate to the directory containing your array image files (.tif) and select the file(s).
4. Click **Add images**.
5. Browse to select the correct design file (.ndf) in the Design File disk provided with your array for the *Design file* field.
6. Browse to select the desired output file destination in the *Choose the destination folder* field.
7. Click **Report**. NimbleScan software creates one Pair report (.pair) for each image file.

Step 7. Analyze Data

NimbleScan software normalizes expression data using quantile normalization as described by Bolstad, et al. (*Bioinformatics* 2003; 19:185).

Gene calls are generated using the Robust Multichip Average (RMA) algorithm as described by Irizarry, et al. (*Nucleic Acids Res*. 2003; 31:e15 and *Biostatistics* 2003; 4:249).

1. After you create pair reports for each image of your experiment, select **Analysis** -> **Expression** -> **RMA Analysis**.

   Note: *RMA analysis must be done with arrays that were made with the same design file.*

2. In *Step 1: Choose the data files to process...*, click **Add files** to add all the .pair files to analyze. All technical replicates (same labeled sample analyzed on arrays made with same design) should be normalized together.
3. In *Step 2: Choose the desired algorithm settings...*, leave the default selections in these fields:
   - *Background correction?* field
   - *Normalize?* field
   - *Save normalized pair data?* field
   - *By container* field

   Refer to the *NimbleScan v2.5 User's Guide* to customize settings.
4. In *Step 3: Choose the destination folder...*, select the desired destination folder for the analyzed data. Roche NimbleGen recommends you create a new folder for each experiment.
5. In *Step 4: Run RMA...*, click **Run RMA**. It may take a few minutes to complete the analysis. The page will return to blank when the analysis is complete.
6. Go to the destination folder that contains the analyzed data and review the files:

File Format	Content	Viewing and Analysis Method
Calls File (_RMA.calls)	Normalized gene expression value and accession number for each gene interrogated by the array	Spreadsheet software (such Microsoft Excel) or ArrayStar or similar microarray gene expression analysis software
Pair File (_norm_RMA.pair)	Identifying information about each probe, their location within the gene, and signal intensity of each probe	Microsoft WordPad

Note: *To generate Non-normalized Calls reports to be used for normalization using different software, select* ***Analysis*** *->* ***Reports*** *->* ***Gene Call*** *in NimbleScan software.*

The .pair and .calls files are compatible with a number of commercially available expression analysis software tools that can be used for further analysis of differentially expressed genes. Go to *www.nimblegen.com/products/software/* for more information.

Notes

Chapter 7. Troubleshooting

This chapter helps you troubleshoot problems that occurred with your microarray experiment.

Sample Quality

Problem	Possible Cause	Recommended Corrective Action
260/230 Absorption Ratio is less than 1.8.	Sample is contaminated with carbohydrate or phenol/chloroform.	Clean up samples using a cleanup column.
260/280 Absorption Ratio is less than 1.8.	Sample is contaminated with protein.	Clean up samples using a cleanup column.
260/280 Absorption Ratio is greater than 2.0.	Sample is contaminated or degraded.	Clean up samples using a cleanup column. Or if degraded, repeat sample extraction.

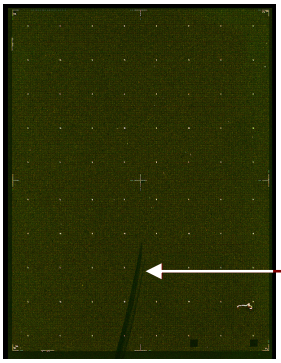
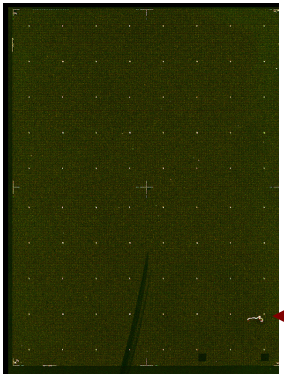
Labeling

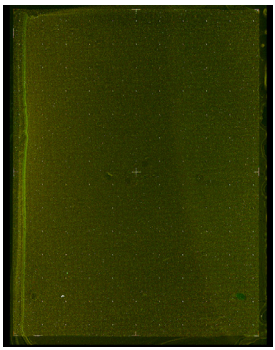
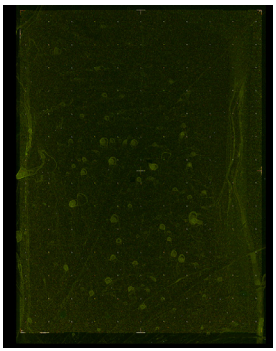
Problem	Possible Cause	Recommended Corrective Action
Labeling yield is less than 6µg per reaction.	Sample is contaminated or degraded.	Check absorption ratios and gel image. If necessary, clean up samples or repeat sample extraction. Repeat labeling.
	Random Primer Buffer is missing ß-Mercaptoethanol or diluted primers, or is older than 4 months. Primers were not diluted correctly.	Check the age of the diluted Random Primer Buffer and that the ß-Mercaptoethanol was added. If necessary, prepare a fresh dilution of nonamer primers. Repeat labeling.
	Klenow enzyme is expired or degraded.	Check the expiration date and follow the labeling kit's storage requirements. Repeat labeling, using fresh enzyme, if necessary.
	Primers are degraded.	Store primers at -20°C, protected from light, and avoid freeze-thaw cycles. Repeat labeling, using fresh primers, if necessary.
	dNTPs are expired or degraded.	Check the expiration date, follow the labeling kit's storage requirements, and avoid freeze-thaw cycles. Repeat labeling, using fresh dNTPs, if necessary.

Hybridization

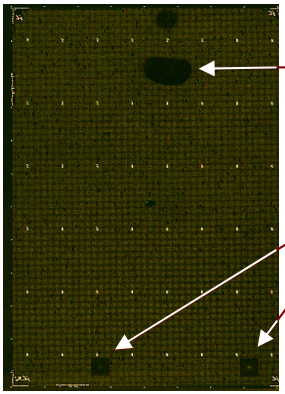
Problem	Possible Cause	Recommended Corrective Action
Mixer is poorly aligned on slide.	The slide was not flush in the PMAT.	Remove the mixer using the Mixer Disassembly Tool then reassemble using a new mixer.
	PMAT is not properly calibrated (multiple mixers misalign).	Send PMAT to Roche NimbleGen for calibration.
Hybridization solution does not enter the hybridization chamber.	The pipette tip is not situated properly on the fill port.	Refer to page 28 for proper loading technique. Ensure that the pipette tip is placed firm and snug against the fill port before dispensing.
Bubbles formed when loading the sample into the mixer's hybridization chamber.	Air was present in the pipette tip.	Use Gilson Positive Displacement Pipettes and follow the instructions on page 28 for proper loading technique. Using the pipette, remove the bubbles and replace with hybridization solution. Remove the bubbles or push them to the corners using the mixer brayer.
Sample leaked out of the mixer before or during the hybridization.	The mixer was not fully adhered to the slide due to incomplete braying.	Refer to page 26 for proper braying technique. Repeat hybridization.
	The mixer port seals/multi-port seals were not fully adhered to the mixer.	Refer to page 29 for proper sealing technique. Ensure that excess hybridization solution has been wiped from the ports before adhering the mixer port seal/ multi-port seal. Repeat hybridization.

Scanning

Problem	Possible Cause	Recommended Corrective Action
Scratches and/or fingerprints are seen on the array image.	The slide was mishandled or dropped.	Grip the slide only on its edges and handle with care.
		
Dust is seen on the array image.	The array was exposed to environmental dust.	If the amount of dust present is small, use the NimbleGen Compressed Gas Nozzle to gently blow compressed inert gas across the array to remove the dust. Rescan the array. If the amount of dust is excessive, repeat wash, dry, and scan.
		
	Dust or dirt was present in the microarray dryer.	Clean the NimbleGen Microarray Dryer or other microarray dryer as instructed in its operator's manual. Repeat wash, dry, and scan.

Problem	Possible Cause	Recommended Corrective Action
Wash artifacts are seen on the array image.  	Slides were not washed and dried completely.	Use the recommended microarray dryer: the NimbleGen Microarray Dryer. Repeat the slide wash, dry, and scan steps, making sure to transfer the slide immediately from the wash solution to the microarray dryer. Blot residual wash buffer from the edges of the slide.
Bright streaks are seen on the array image.	The microarray dryer does not accelerate fast enough.	Ensure proper function and maintenance of the microarray dryer. The microarray dryer should achieve a top speed of at least 1,400rpm in a minimum of 0.8 seconds.
Part of the array is missing from the array image.	The scan area is not specified properly.	Refer to page 36 for instructions on how to specify the scan area. Repeat the scan ensuring that fiducial features are included in the scan area.
The array image appears dim.	PMT settings are not adjusted correctly.	Refer to page 36 for instructions on how to adjust PMT settings. Repeat scan.
	Hybridization conditions are too stringent.	Check that the hybridization solution was prepared correctly (refer to page 24) and the NimbleGen Hybridization System is set to and maintaining 42°C. Repeat hybridization.
	Sample leaked out of the mixer during hybridization due to incomplete braying.	Refer to page 26 for proper braying technique. Repeat hybridization.

Problem	Possible Cause	Recommended Corrective Action
The array image appears dim. *(continued)*	Cy dye is degraded due to exposure to light, ozone, and/or humidity.	Store primers at -20°C, protected from light. Maintain ozone levels below 20ppb and humidity levels below 40%. Repeat hybridization.
The fiducial features appear dim or blank.	The alignment oligo was either not added to the hybridization solution or was degraded due to repeated freeze-thaw cycles.	Repeat hybridization, using fresh alignment oligo, if necessary.
Features appear blank on portions of the array.	The slide contains a scratch or fingerprint.	Grip the slides only on the edges and handle with care. Repeat hybridization.
	One or more bubbles were present in the hybridization chamber.	Repeat hybridization if blank regions cover greater than 5% of the array area.
	Wash buffer dried onto the array surface in between wash steps.	Ensure that slides are transferred quickly between wash steps. Repeat hybridization.



Problem	Possible Cause	Recommended Corrective Action
The array image is too bright.	Hybridization conditions are not stringent enough.	Check that the hybridization solution was prepared correctly (refer to page 24) and the NimbleGen Hybridization System power is on, mixing is on, and temperature is set to and maintaining 42°C. Repeat hybridization.
	PMT settings are not adjusted correctly.	Refer to page 36 for instructions on how to adjust PMT settings. Repeat scan.
The array image brightness is uneven.	One or more bubble(s) were present in the hybridization chamber, and/or there was poor mixing during hybridization.	Repeat hybridization. If the problem persists, refer to the *NimbleGen Hybridization System User's Guide* for troubleshooting information.
	Sample leaked out of the mixer during hybridization due to incomplete braying	Refer to page 26 for proper braying technique. Repeat hybridization.
	The sample pellet was not properly rehydrated or mixed with the hybridization solution.	During sample preparation, be sure to vortex and spin the sample before and after the 95°C incubation. Repeat hybridization.
	Array washing was not done properly.	Refer to page 30 for proper washing technique. Repeat hybridization.
Features appear out of alignment.	Scanner is out of alignment.	Refer to the scanner's documentation. Scanner calibration/service is recommended.

Sample Tracking Controls (STCs)

Problem	Possible Cause	Recommended Corrective Action
STC features are not visible in the STC control regions that are located along the perimeter, in the upper left corner, and in the center of the array.	Each sample was not resuspended in an STC, or the STC was degraded from repeated freeze-thaw cycles.	Repeat hybridization, using a fresh STC, if necessary.
STC features representing multiple STCs are visible on the array image or reported in the Sample Tracking report.	Sample integrity was compromised during sample preparation, loading, or hybridization.	Repeat hybridization, ensuring the following: ■ The mixer is fully adhered to the slide before loading sample. Refer to page 26 for proper braying technique. ■ Excess sample is removed from around the loading ports. Refer to page 29 for proper sealing technique. ■ The mixer port seals/multi-port seals were not fully adhered to the mixer after loading sample. Refer to page 29 for proper sealing technique.

Data Analysis

Problem	Possible Cause	Recommended Corrective Action
NimbleScan fails to open the image TIFF file or Multiplex images fail to burst.	The wrong .ncd file was specified.	Refer to page 41 for instructions on how to specify the correct .ncd file when bursting multiplex arrays.
	The image was corrupted.	Rescan array.
	A non-16-bit TIFF image was specified.	Rescan and save as a 16-bit TIFF image. NimbleScan software reads only 16-bit grayscale images.
Multiplex images were burst incorrectly.	The entire array area was not scanned.	Refer to page 36 for instructions on how to specify the scan area. Check the image in GenePix software and rescan.
	The scanned area is too large.	Refer to page 36 for instructions on how to specify the scan area. Crop the image in NimbleScan software and attempt bursting again.
	The array area is not centered in the scanned image.	Refer to page 36 for instructions on how to specify the scan area. Crop the image in NimbleScan software or rescan if necessary.
The auto align function fails to grid the array image.	Fiducial features are dim.	Perform a manual alignment as described in the *NimbleScan User's Guide*.
	NimbleScan v2.4 or higher was not used.	Install the latest version of NimbleScan software. Reload the image and perform the auto align function.
The auto align function improperly grids the array.	Bright artifacts are present in the corners of the array image.	Perform a manual alignment as described in the *NimbleScan User's Guide*.

Notes

Appendix A. Expression Data Analysis

You can review the resultant pair and calls files from an RMA analysis and analyze the expression profile of each sample. This appendix briefly describes how to use the ArrayStar software to import normalized data and perform a variety of analytical and visualization techniques.

Step 1. Install the Necessary Software

Download a free, fully functional trial version of the DNAStar ArrayStar software at *http://nimblegen.dnastar.com/forms/demo_requestnimble.php*.

Step 2. Import Your Expression Data

1. Open ArrayStar software.
2. To import .calls files:
   a. Select **File** -> **Import Experiments**.
   b. Click **Browse** to find and select the .calls files to import.
3. To import gene annotations:
   a. Select **File** -> **Import Annotations**.
   b. Click **Browse** to find and select the .ngd files to import.
4. Select **File** -> **Save Project** to save your files as an ArrayStar project (.dmaproj).

Step 3. Analyze Your Expression Data

There are many techniques for analyzing microarray expression data from your ArrayStar project. Following is a description of some options supported by the ArrayStar software.

1. To view a pair-wise comparison in the Scatter Plot view:

   a. Select **Graphs -> Scatter Plot**.

      Figure 21 shows example scatter plots for data from two technical replicates (panel A) and for data from two different tissue samples (panel B). The statistics panel from the Scatter Plot Info Pane provides information on the correlation between the samples (r^2) and the number of genes present at specified fold changes between samples.

   b. Click the link for a specific fold change to select the genes for further analysis.

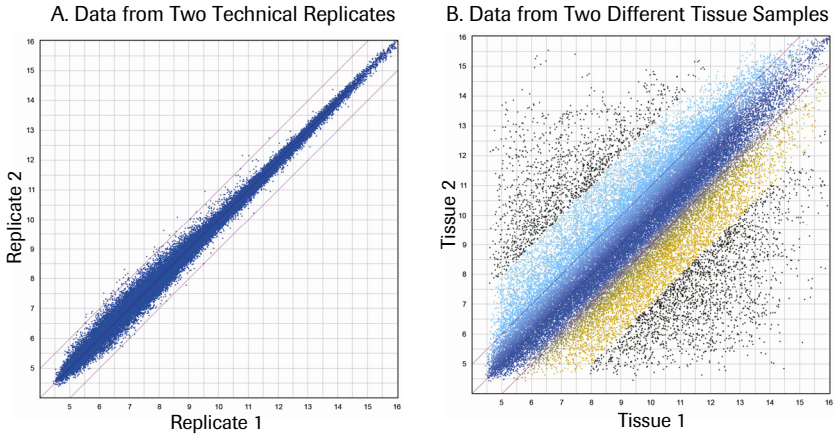


Figure 21: Scatter Plots

2. To view a Hierarchical clustering of your data set in the Heat Map view:

   a. Select **Clustering** -> **Hierarchical**.

      Figure 22 shows an example heat map for the genes expressed at ≥ 8-fold difference between the samples shown in Figure 21B.

   b. Select genes of interest by clicking on a node in Gene Tree, shown on the Y axis of the Heat Map, and then select **Data** -> **Show Gene Table**.

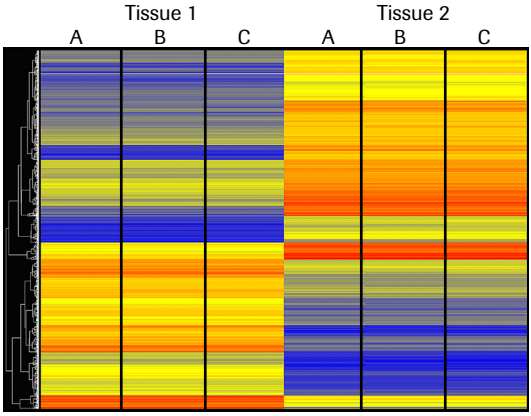


Figure 22: Heat Map

3. To cluster the selected genes with the k-Means method:

   a. Select **Clustering** -> **k-Means**.

   b. Display your results in the Line Graph Thumbnails view.

   c. Double-click a graph in the Line Graph Thumbnails view to launch the Line Graph view for the cluster you selected.

4. To view detail information for each gene:

   a. Select **Data** -> **Show Gene Table**.

   b. Add columns to the table using the buttons in the Gene Table toolbar.

5. Select **File** -> **Save Project** to save your work.

Notes

Appendix B. Limited Warranty

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NIMBLEGEN ARRAYS

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Roche NimbleGen, Inc.
504 S. Rosa Road
Madison, WI 53719 USA